

*Amendment and Response Under 37 C.F.R. §1.116 - Expedited Examining Procedure*

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*Serial No.: 09/600,392*

*Confirmation No.: 4850*

*Filed: September 8, 2000*

*For: AN AUTOREGULATORY SYSTEM FOR VALIDATING MICROBIAL GENES AS POSSIBLE  
ANTIMICROBIAL TARGETS USING A TETRACYCLINE-CONTROLLABLE ELEMENT*

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### Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

1. **(Currently amended)** A process for the identification of a microbial gene encoding a gene product that is important to a microbe's ability to infect or sustain an infection in a mammal, which process comprises:

    infecting a plurality of mammals with a microbe that has been genetically altered such that the amount of said gene product produced by said genetically altered microbe is regulated by a Tetracycline-Controllable Element (TCE);

    where said TCE is a gene regulatory system that controls the expression of the target gene product through its ability to modulate the function of said gene in response to said microbe's exposure to tetracycline, and where said TCE is comprised of a tetracycline-controllable transcription promoter polynucleotide sequence;

    where said genetically altered microbe also comprises a polynucleotide sequence encoding a tetracycline resistance protein;

    where said polynucleotide sequence encoding a tetracycline resistance protein is contained on a tetracycline resistance and repressor DNA cassette (TRRDC), said TRRDC comprising a tetracycline repressor gene and a tetracycline resistance gene;

    where said TCE is operably linked to both a first polynucleotide sequence encoding a reporter gene (RG) and a second polynucleotide sequence comprising a target gene (TG);

    exposing the plurality of mammals to tetracycline;

    once an infection with the genetically altered microbe is established, removing the tetracycline exposure of a portion of the plurality of mammals, such that a first group of the plurality of mammals is exposed to tetracycline and a second group of the plurality of mammals is not exposed to tetracycline; and

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comparing the degree of infection, microbe levels, or survival rates of the mammals in the first group and the second group wherein a difference between the two groups of animals in the survival rates, levels of microbes, or levels of infection present identifies the gene product as important to a microbe's ability to infect or sustain an infection in a mammal.

2. (Canceled)

3. (Previously presented) The process of claim 1, where said tetracycline-controllable transcription promoter polynucleotide sequence is a prokaryotic transcription promoter.

4. (Canceled)

5. (Previously presented) The process of claim 1, where said reporter gene encodes a  $\beta$ -lactamase.

6. (Canceled)

7. (Previously presented) The process of claim 1, where the TCE, the TRRDC, the RG, and the TG are all on the same DNA cassette, referred to as a Regulatory DNA Cassette (RDC).

8. (Previously presented) The process of claim 1, where said TRRDC promoter is operably linked to the TCE, the tetracycline repressor gene comprises the structural gene *tetM*, and the tetracycline resistance gene comprises the structural gene *tetR*.

9. (Previously presented) The process of claim 1, where said difference between the two groups of animals is a difference in the levels of microbes or levels of infection present in the mammals.

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10. (Previously presented) The process of claim 1, where said difference between the two groups of animals is a difference in the survival rates of the groups of animals.
11. (Previously presented) The process of claim 1, where said difference between the two groups of animals shows that animals exposed to tetracycline have poorer health, higher rates of infection, lower survival or higher levels of microbes than animals not exposed to tetracycline.
12. (Previously presented) The process of claim 1, where said tetracycline resistance gene of said TRRDC comprises sequences from the *Staphylococcus aureus tetM* gene.
13. (Previously presented) The process of claim 1, where said tetracycline repressor gene of said TRRDC is obtained from the Tn10 transposon.
14. (Previously presented) The process of claim 1, where said TRRDC comprises the sequence of SEQ ID NO:35 or SEQ ID NO:36.
15. (Previously presented) The process of claim 1, where said infected mammals are mice.
16. (Previously presented) The process of claim 1, where said genetically altered microbe is a *Staphylococcus* species.
17. (Previously presented) The process of claim 16, where said *Staphylococcus* species is *Staphylococcus aureus*.
18. (Previously presented) The process of claim 1, where said microbe is a virus.

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19. (Previously presented) The process of claim 1, where said microbe is a lower eukaryote.

20. (Previously presented) The process of claim 1, where said microbe is a yeast.

21.-76. (Canceled)

77. (Currently amended) A process to regulate expression of a gene product by a microbe in a mammalian host with tetracycline or a tetracycline analog, said process comprising:

infecting a mammalian host with a microbe that has been genetically altered such that the amount of said gene product produced by said genetically altered microbe is regulated by a Tetracycline-Controllable Element (TCE);

where said TCE is a gene regulatory system that controls the expression of the target gene product through its ability to modulate the function of said gene in response to said microbe's exposure to tetracycline, and where said TCE is comprised of a tetracycline-controllable transcription promoter polynucleotide sequence;

where said genetically altered microbe also comprises a polynucleotide sequence encoding a tetracycline resistance protein;

where said polynucleotide sequence encoding a tetracycline resistance protein is contained on a tetracycline resistance and repressor DNA cassette (TRRDC), said TRRDC comprising a tetracycline repressor gene and a tetracycline resistance gene;

where said TCE is operably linked to both a first polynucleotide sequence encoding a reporter gene (RG) and a second polynucleotide sequence comprising a target gene (TG); and exposing the mammalian host to tetracycline.

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78. **(Previously presented)** The process of claim 77, further comprising, once an infection with the genetically altered microbe is established, removing the tetracycline exposure of the mammalian host.

79. **(Previously presented)** The process of claim 1, where said plurality of mammals are exposed to tetracycline while being infected with the genetically altered microbe.

80. **(Previously presented)** The process of claim 1, where said plurality of mammals are exposed to tetracycline by adding tetracycline to the drinking water.

81. **(Canceled)**

82. **(Previously presented)** The process of claim 77, where the TCE, the TRRDC, the RG, and the TG are all on the same DNA cassette, referred to as a Regulatory DNA Cassette (RDC).